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Electrophysiology and Innervation of the Photosensitive Epistellar Body in the Lesser Octopus *Eledone cirrhosa*

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Abstract. The innervation and responses to light of the cephalopod epistellar body were investigated in preparations isolated from the stellate ganglia of the lesser or northern octopus, *Eledone cirrhosa*. Extracellular generator potentials in response to flashes of light were recorded from these photosensitive vesicles, with the amplitude of the response being found to be dependent upon the intensity of the flash and the level of ambient illumination. Intracellular recordings from photoreceptor cells of the epistellar body showed that they had resting potentials of about -49 ± 7 mV (mean $\pm$ SD, $n = 43$) and were depolarized by flashes of white, but not red (>650 nm) light. The evoked depolarization consisted of a transient component, followed by a steady plateau in which the amplitude of the depolarization was well correlated with the log of the stimulus intensity. The evoked depolarizations induced action potentials in the photoreceptor cells, with the frequency of firing being well correlated with the stimulus intensity. The morphologies of individual photoreceptor cells were visualized by intracellular injections of the fluorescent dye Lucifer yellow, and the path of the epistellar nerve across the stellate ganglion, into the pallial nerve, toward the brain was traced using the lipophilic dye Di-1. This pathway was confirmed physiologically by recording light-evoked responses from the cut end of the pallial nerve.

Introduction

Most cephalopods have, in addition to their retinal photoreceptor system, extraocular photoreceptors or photosensitive vesicles (PSVs). The PSVs of octopods such as *Eledone moschata* are located inside the mantle sac and appear as a small pigmented vesicle on the ventral posterior margin of the stellate ganglion (Bauer, 1909; Young, 1936, 1971; reviewed in Mauro, 1977; Fig. 1). These PSVs have also been termed the 'epistellar bodies' (Young, 1929, 1936). The ultrastructure of the epistellar body of *E. moschata* shows packed arrays of photoreceptor cells with microvilli, reminiscent of rhabdomeres, but without dioptric apparatus (Nishioka *et al.*, 1962), together with an epistellar nerve running into the stellate ganglion (Young, 1936; Perrelet and Mauro, 1972). The epistellar photoreceptor system in *E. moschata* has been shown to contain the visual pigment rhodopsin, with a maximum absorption wavelength of 475 nm; this is very close to 470 nm, which is the maximum absorption wavelength of retinal rhodopsin in this octopod species (Nishioka *et al.*, 1966; Mauro, 1977). This wavelength is also close to electrophysiological estimates of spectral sensitivity in *E. moschata* (Nishioka *et al.*, 1966; Mauro, 1977). The accessory pigment retinochrome is also present in cephalopod PSVs (Hara and Hara, 1980; Ozaki *et al.*, 1983). Although preliminary evidence has indicated that the photoreceptor cells within the epistellar body of octopus give depolarizing responses to light (Mauro and Baumann, 1968; reviewed in Mauro, 1977), there has been no detailed study of these responses.

Previously we have shown that extracellular, light-induced generator potentials in the epistellar bodies of *Eledone cirrhosa* can be produced by light flashes of different intensity and duration, transmitted through the mantle sac wall of the octopus (Cobb *et al.*, 1995a,b). In addition, the normal circadian behavioral rhythm entrained by a

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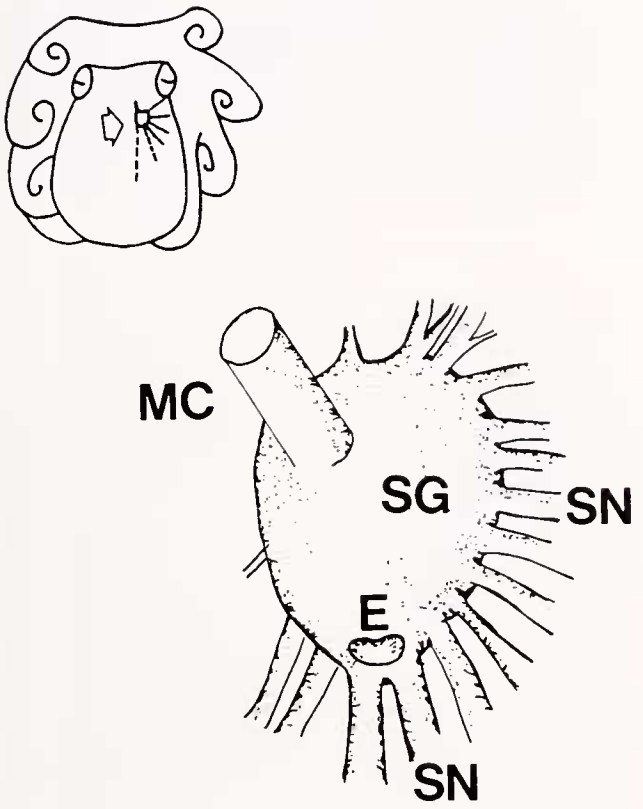

Figure 1. Diagrams showing the position of the photosensitive vesicle or epistellar body in an octopus (not to scale). Top left: dorsal right view of octopus showing position of stellate ganglion (arrow) attached to the inner surface of the dorsal mantle sac wall. Bottom right: ventral view of left stellate ganglion in the same orientation as top left, with epistellar body (E), stellate ganglion (SG), stellar nerve (SN), and mantle connective or pallial nerve (MC) indicated.

light-dark cycle is maintained after removal of the epistellar body in sighted octopus (Cobb *et al.*, 1995a,b). In the present study, we examine the capacity of the octopod extraocular photoreceptor system for light detection by characterizing the extracellular and intracellular responses to light flashes of increasing intensity and duration in the octopus *Eledone cirrhosa*. Lucifer yellow was used to stain iontophoretically and identify the photoreceptor cells in the octopus epistellar body; there was no evidence for dye coupling between these extraocular photoreceptor cells. It is also established that the epistellar body sends afferent nerves that pass across the stellate ganglion and connect with the central nervous system, *via* the mantle connective (pallial) nerve.

Materials and Methods

Collection and maintenance of experimental animals

The lesser or northern octopus, *Eledone cirrhosa* (dorsal mantle length 5–14 cm) used in this study were caught offshore near the coast of Plymouth, UK, at a depth of 10–15 m and transported to laboratory holding tanks with flow-through, aerated seawater at 12°–18°C. To prevent the octopus from escaping, the side walls of the tank were lined with 1-cm-thick sheets of plastic foam (Boyle, 1981). Octopus were supplied with live crab (*Carcinus maenus*) food *ad libitum* and were maintained in the tank system for up to 9 months until required.

Electrophysiology

For electrophysiological recordings, an octopus was anesthetized with 3% ethanol in seawater and decapitated. The paired stellate ganglia and attached epistellar bodies were then removed from the dorsal mantle wall. The epistellar body and attached stellate ganglion were washed in fresh seawater and pinned out, ventral side up, in a Sylgard-lined recording dish filled with filtered artificial seawater (ASW: NaCl 470 m*M*, KCl 10 m*M*, $MgCl_2$ 50 m*M*, $CaCl_2$ 10 m*M*, MOPS (3-[N-Morpholine]propane-sulfonic acid) 10 m*M*, pH adjusted to 7.8, osmolarity 1010 mmol/kg). All subsequent electrophysiological recordings were made at room temperature (between 18° and 23°C).

Illumination stimuli were provided by a Schott KL1500 cold light source, with a quartz halogen bulb (Thorn EMI 15 V, 150 W). The light was passed through a standard heat filter into a glass fiber light guide and then, *via* an electronically controlled shutter and a second fiber light guide, to the ventral side of the stellate ganglion and epistellar body. The duration of the light flash was set by the electronic shutter (Uniblitz TI32, Optilas Ltd, UK) to between 10 ms and 10 s. The light intensity was varied by inserting neutral density filters into the light path and was measured at the preparation level using a portable calibrated radiometer (Ealing Electro-optics, UK: model 27-5479). An additional, uncalibrated photocell was used to record the precise timing and duration of the stimulus light flashes. In some experiments, a red filter (Kodak 1A, UK, wavelength >650 nm) was introduced into the light path. Suction electrodes were used to take extracellular recordings from the mantle connective (pallial) nerve of the stellate ganglion in response to flashes of white or red light that were applied to the epistellar body of the preparation from two octopus.

The extracellular receptor potentials were recorded by inserting a low-resistance microelectrode (2 MΩ resistance when filled with ASW) into the epistellar body wall. Intracellular recordings from photoreceptor cells in isolated intact epistellar bodies were made using high-resistance microelectrodes of borosilicate glass capillaries with inner filaments (Clark Electrochemical, UK, GC-150F, 1.5 mm OD × 0.86 ID), filled with 3 *M* KCl and having tip resistances of 30–150 MΩ. A conventional

microelectrode amplifier (AxoClamp 2B amplifier, Axon Instruments, Inc. USA) was used for recording resting, generator, and receptor potentials and for injecting current pulses through the intracellular microelectrode. In some experiments, octopus photoreceptor cells were injected iontophoretically with the fluorescent napthalimide dye Lucifer yellow CH (Stewart, 1978; Sigma, UK). For this the microelectrodes were back-filled with 3% Lucifer yellow in 1 *M* LiCl and had resistances of 150–200 MΩ. Injections of hyperpolarizing current (1.0 nA at 1 Hz) resulted in rapid movement of Lucifer yellow into the cells, and dye filling was considered complete after 30 min. Resting potential was recorded during cell impalement and generator potentials were recorded in response to light flashes, before switching to current injection in these experiments. Immediately after dye injection, the preparation was photographed in whole mount under an epifluorescent microscope, using color film (400 ASA).

For normal recordings, the signal from the microelectrodes was amplified and, together with the signal from the photocell monitor, was passed to a computer-controlled signal averager (CED 1401 computer interface running Sigavg software, Cambridge Electronic Design, UK). Typically, 5–10 responses were averaged to improve signal-to-noise ratios, and repeated light flash stimuli were separated by at least 20 s. The octopus preparations remained viable for at least 6 h. Illumination of the experimental 'darkroom' was provided by red safelight (>650 nm). Illumination of 0.1 $\mu W/cm^2$ with this red light was found not to cause a decrement in photoresponse.

Epistellar body innervation

Innervation of the epistellar body was studied by orthodromic filling from the epistellar body using the lipophilic dye Di-I (Honig and Hume, 1989) in 10 animals. With the aid of a dissecting microscope, the ASW was removed from the dish and a crystal of the fluorescent carbocyanine lipophilic dye Di-I (1,1′-dioctadecyl-3,3,3′,3′ tetramethylindocarbocyanine perchlorate, D-282 (Di-I C18(3)), Molecular Probes, Inc, USA) was placed inside the epistellar body of each stellate ganglion through an incision made with a fine steel pin or razor blade. The tissues were then fixed in 2.5% paraformaldehyde in 0.1 *M* phosphate buffer. A microscope with epifluorescence and a rhodamine filter (Nikon DM580) was used to map the progress of the orange Di-I fluorescence from the epistellar body, across the stellate ganglion, through axons and into the pallial nerve over several days. Tissues were cleared in 60% (w/v) meglumine lothalamate with 0.01% (w/v) sodium calciumedetate and 0.01% (w/v) sodium acid phosphate (product: Conray 280, May & Baker Ltd (Rhone-Poulenc Group), UK; Zill *et al.*, 1993), before the Di-I stained axons were photographed (400 ASA Kodak black-and-white film), under the epifluorescence microscope with rhodamine filter set. Additional white light illumination was sometimes used to observe and photograph the relative positions of fluorescent Di-I-filled axons and the stellate ganglion tissue.

Results

Anatomy and innervation of the octopus epistellar body

Figure 2 shows the size, shape, and position of the epistellar body that contains the extraocular photoreceptors on the stellate ganglion of *Eledone cirrhosa*. The epistellar body is located at the ventral posterior margin of the ganglion and is commonly spherical (Fig 2A), although it sometimes appears divided into two or more compartments (Fig 2B). It is confined within the common capsule of connective tissue surrounding the ganglion, but is separated from the ganglionic neuronal cell bodies by one or more sheath layers. The epistellar body is usually orange in freshly dissected preparations, but the color may fade with time after dissection or be absent in some animals, particularly larger, older specimens.

In the course of the intracellular recordings described below, some photoreceptor cells (n = 4) were injected

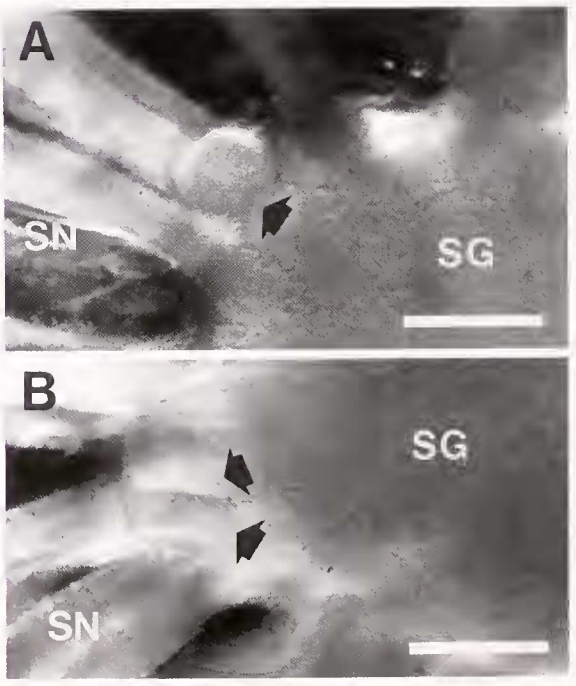

Figure 2. Epistellar body (arrows), stellate ganglion (SG), and stellar nerves (SN) of the octopus *Eledone cirrhosa*. The epistellar body is commonly spherical (A), but sometimes appears to be divided into two compartments (B). Scale bars are 1 mm.

with Lucifer yellow, a fluorescent dye (Fig. 3). The dye fills showed that the photoreceptors have a cell soma (50 μm) that is located in the wall of the epistellar body and gives rise to a single axon, extending towards the periphery of the epistellar body. The axons from separate photoreceptor cells converge outside the epistellar body to form the 'epistellar' nerve. In addition, a process that may be as long as 100 μm projects towards the center of the epistellar body, sometimes dividing or branching toward its distal end. No dye coupling between photoreceptors was observed in any of the cells that were well filled with dye.

Figure 4A shows an epistellar body, the attached stellate ganglion, and the connection of the mantle connective (pallial) nerve to the ganglion. For all cases (n = 8) in which the epistellar body and attached stellate ganglion preparations were filled with fluorescent Di-I (Fig. 4B, C, D), orthodromic fills indicated that the epistellar body gave off an 'epistellar' nerve (Fig. 4B, C, D) that passed across the stellate ganglion, into the mantle connective (pallial) nerve (Fig. 4B, C), and then, presumably, to the brain. The nerve bundle was more shallow in its path across the stellate ganglion in some preparations (n = 2) than in others. In these preparations, the epistellar nerve remained intact and branched, sometimes into three separate fiber bundles (Fig. 4B), only before entry to the mantle connective (pallial) nerve. In addition, these preparations showed a single nerve branch, which occurred close to the epistellar body. This branch separated from the epistellar nerve and passed deep into the stellate ganglion, to an unidentified destination (Fig. 4B). However, in most preparations examined (n = 8), the Di-I-filled epistellar nerve branched close to the epistellar body, forming numerous nerve bundles that passed into the mantle connective nerve and then toward the central nervous system (Fig. 4C, D). In addition to the epistellar nerve, these preparations showed two Di-I-filled nerves running from the side of the epistellar body in parallel to and on each side of the epistellar nerve (Fig. 4C, D). These parallel

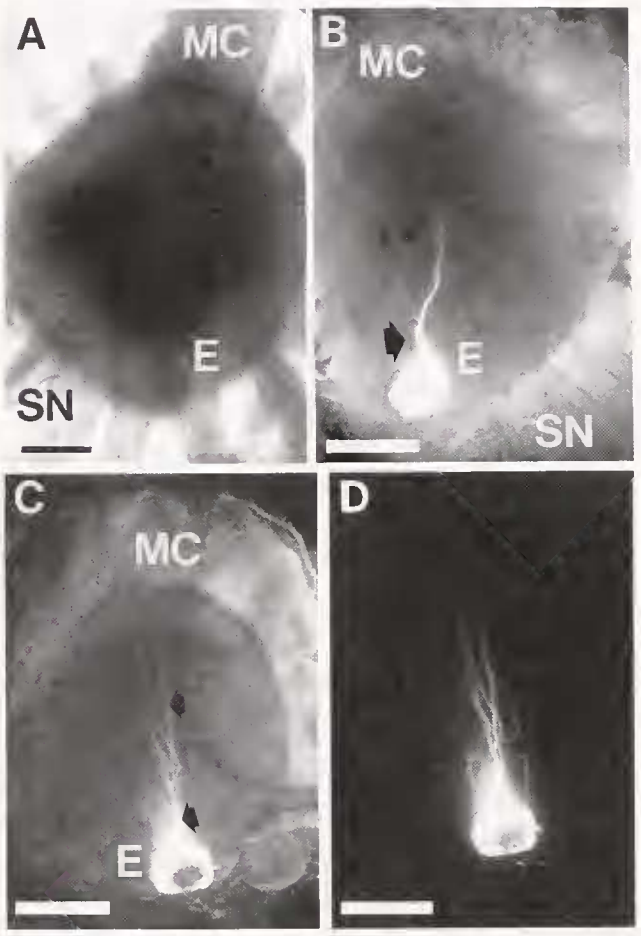

Figure 4. Innervation of the epistellar body. (A) Epistellar body and stellate ganglion of the octopus *Eledone cirrhosa*. The ovoid epistellar body (E) lies at the posterior end of the stellate ganglion near the origin of several stellar nerves (SN), opposite the mantle connective (pallial) nerve (MC). Scale bar is 1 mm. (B) Whole mount of epistellar body and stellate ganglion with orthodromic fluorescent Di-I-filled 'epistellar' nerve (arrow), epistellar body (E), stellar nerves (SN), and mantle connective (pallial) nerve (MC). Scale bar is 1 mm. (C) Whole mount of stellate ganglion with orthodromic fluorescent Di-I-filled epistellar body photoreceptor cell axons (arrows) connecting the epistellar body (E) and the mantle connective (pallial) nerve (MC). Scale bar is 1 mm. (D) Whole mount as in (C) under rhodamine-filtered light only, showing detail of Di-I-filled axons. Scale bar is 1 mm.

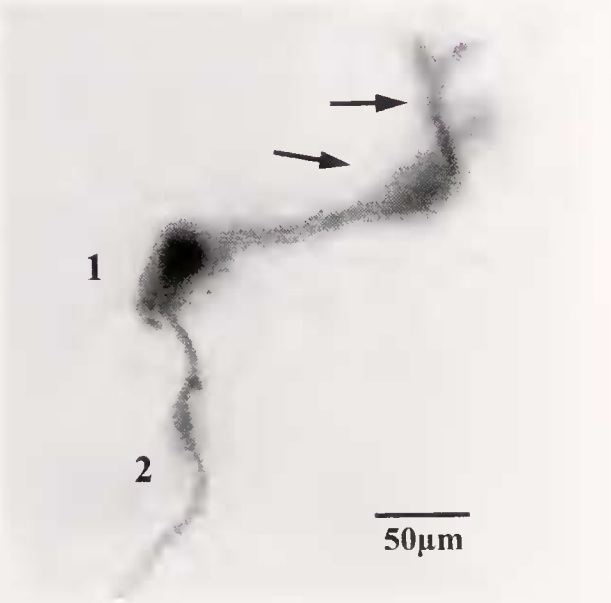

Figure 3. Iontophoretic microinjection of Lucifer yellow into an epistellar body photoreceptor cell. Detail of photoreceptor cell filled with Lucifer yellow showing cell soma (1) and axon process (2). Note long, branched process (arrows) that extends toward the center of the epistellar body.

nerve bundles then appeared to focus on the central path of the epistellar nerve bundles and pass into the center of the stellate ganglion (Fig. 4C, D), perhaps to the neuropil. The destination remains conjecture, however, because none of the preparations examined showed conclusive evidence of interaction between a Di-1-filled nerve fiber and the neuropil of the stellate ganglion.

Recordings from the mantle connective (pallial) nerve

To test whether light-evoked activity from the epistellar body could be observed in the mantle connective (pallial) nerve, suction electrode recordings were made from the cut end of the nerve as controlled light flashes were applied to the epistellar body. When white light was flashed, a compound action potential was observed, mainly comprising a large downward trough (Fig. 5). To test for artifacts and to determine the spectral sensitivity of the suction electrode response, a flash stimulus of red light was produced by using a >650 nm filter. Whereas a flash of white light evoked an extracellular voltage response in the mantle connective (pallial) nerve, a flash of red light, of the same duration, evoked no response (Fig. 5). The relatively long delay between stimulus and response does not permit any conclusions to be drawn about whether this is a direct or post-synaptic response; clearly the former would be expected.

Electrophysiology of the epistellar body photoreceptors

Generator potential responses induced by light flashes of constant duration but increasing intensity were recorded extracellularly from the epistellar body photoreceptors of the octopus, *Eledone cirrhosa* (Fig. 6A). The generator potential appeared as a short-latency downward deflection of the voltage trace, and the amplitude of the evoked response increased with increasing flash intensity. A graph of response against stimulus intensity (Fig. 6B) shows that the amplitude of the evoked response was well correlated to the log of light intensity across the entire range examined (Fig. 6B). The latency between the start

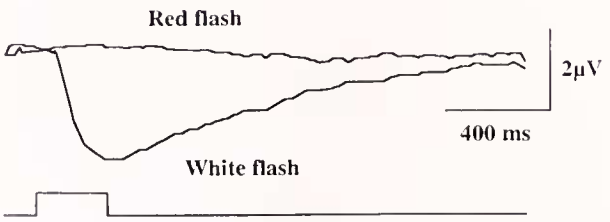

Figure 5. Light-evoked extracellular responses in the mantle connective (pallial) nerve of the stellate ganglion preparation recorded by suction electrode in response to flashes of white and red light. Duration of the light flash applied to the epistellar body is indicated in the bottom trace.

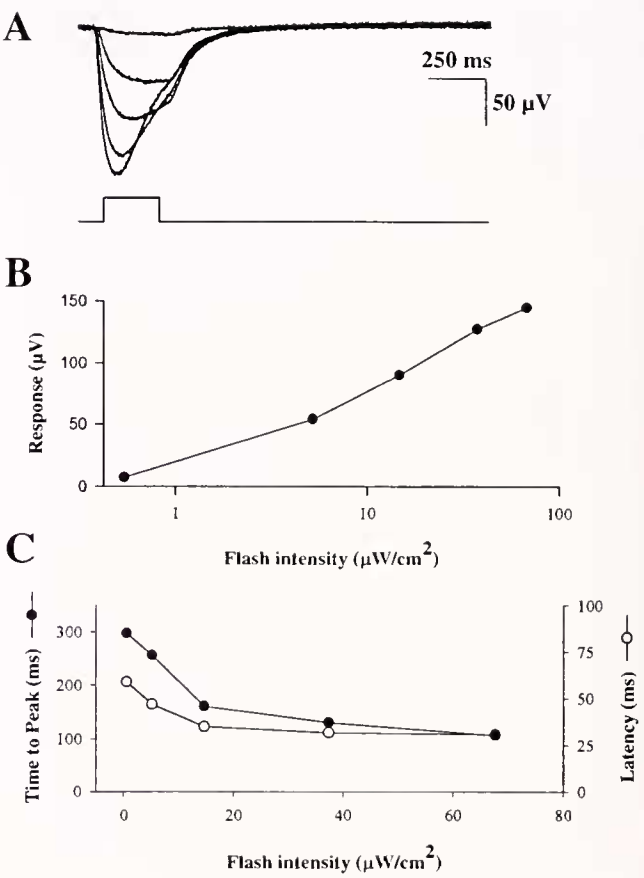

Figure 6. Epistellar body responses to change in intensity of the light stimulus. (A) Upper traces show generator potentials recorded extracellularly from photoreceptor cells in the epistellar body in response to a series of 250-ms-duration light flashes, as indicated in the lower trace, of increasing intensity. (B) Graph of log light-flash intensity against extracellular response amplitude. (C) Latency and rise time of the extracellular response with increasing stimulus intensity.

of the stimulus and the start of the photoreceptor response was between 40 and 25 ms, and this latency decreased with increased stimulus intensity (Fig. 6C). Increasing stimulus intensity was also linked with a decrease in the time from the start of the stimulus to the peak of the response or rise time (Fig. 6C). The epistellar photoreceptor cells needed some time to recover from each light flash: the extracellular response generated by a second flash given 2.5 s after the first was reduced by almost 80% (Fig. 7A). This decrease in response was evident up to 30 s after the first light flash. A plot of the response amplitude to the second flash (Fig. 7B) showed that the recovery had an exponential time curve with a time constant of almost 6 s. Similarly, the size of the extracellular response to a single light flash was affected by the intensity level of ambient illumination (Fig. 8). Figure 8A shows the extracellular responses to constant light flashes given after at least a 1-min exposure to three different

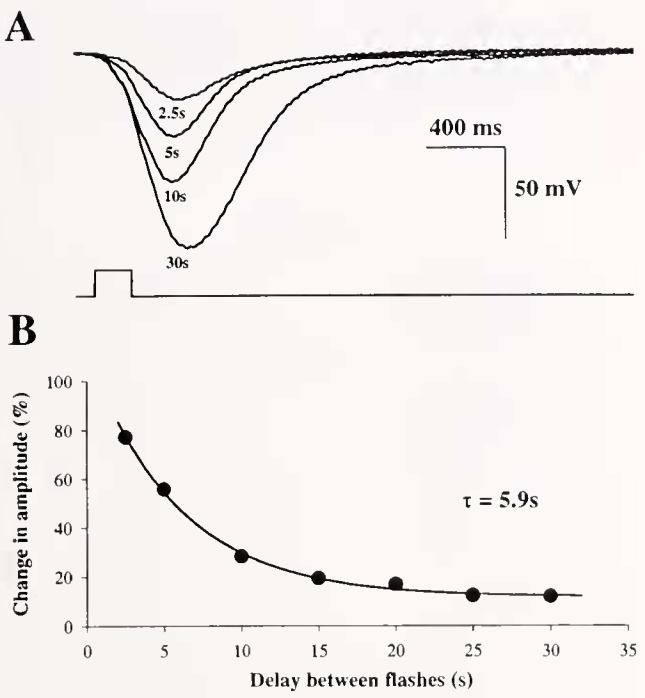

Figure 7. Adaptation or recovery responses between light flashes to the epistellar body. (A) Upper traces show examples of photoreceptor cell generator potentials recorded extracellularly from the octopus epistellar body in response to a series of 200-ms-duration light flashes with increased delay between flashes, but constant intensity (34 $\mu W/cm^2$), as indicated in the lower trace. (B) Amplitude of the extracellular response with increasing delay between flashes.

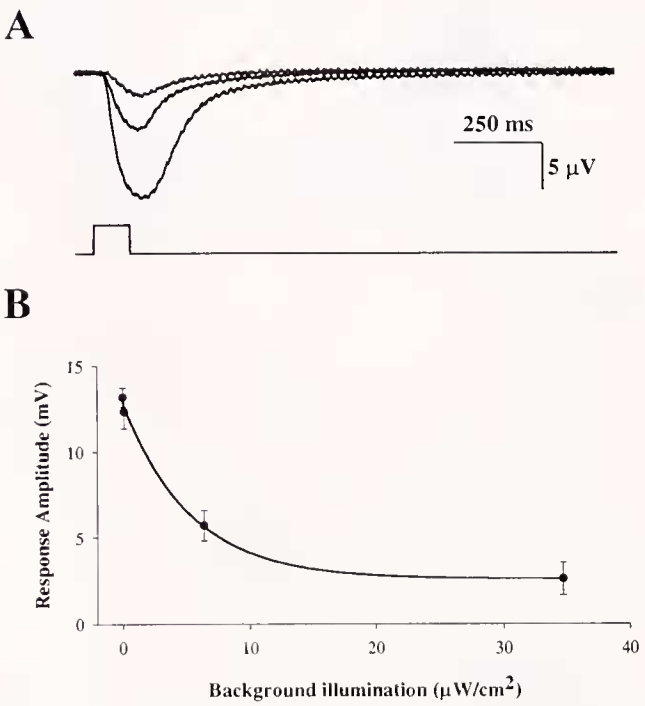

Figure 8. Effect of background illumination on the response of the epistellar body to light-flash stimuli. (A) Upper traces show examples of photoreceptor cell generator potentials recorded extracellularly from the octopus epistellar body in response to a 200-ms-duration light flash (intensity 167 $\mu W/cm^2$), as indicated in the lower trace, while subjecting the epistellar body to increased background illumination. (B) Amplitude of the extracellular response with increasing background illumination. In all cases each point represents the mean ± SEM, n = 10 separate receptor responses.

intensity levels of 'background' illumination. It can be seen that the greater the level of background illumination, then the smaller the extracellular response to the light flash; Figure 8B shows that the relationship is more-or-less exponential.

Intracellular recordings from individual photoreceptor cells demonstrated that, in the dark, these had membrane resting potentials of about −49 ± 7 mV (mean ± SD, n = 43); such recordings could be maintained for up to 45 min. Epistellar body photoreceptors responded to a short flash of white light with a depolarization that often resulted in the firing of a burst of action potentials (Fig. 9). Although not studied in detail, this response was dependent on the wavelength of the light stimulus, for stimuli at wavelengths greater than 650 nm evoked no response (Fig. 9). This also provided confirmation that the response was not artifactual or related to other stimuli, such as the noise of the mechanical shutter. The amplitude of the intracellular receptor potential response varied with the intensity of the light flash (Fig. 10A). A graph of these responses (Fig. 10B), demonstrated that the amplitude of the depolarization was well correlated to the log of the light-flash intensity across the intensity range examined. Similarly, Figure 10B illustrates that the peak firing frequency of the resulting action potentials was well correlated to the log of the stimulus intensity and the conversion factor from receptor potential to action potentials or 'spikes' is about 2 spikes/mV depolarization; that value is within the range of other invertebrate spike encoding photoreceptor cells—for example 1 spike/mV in eccen-

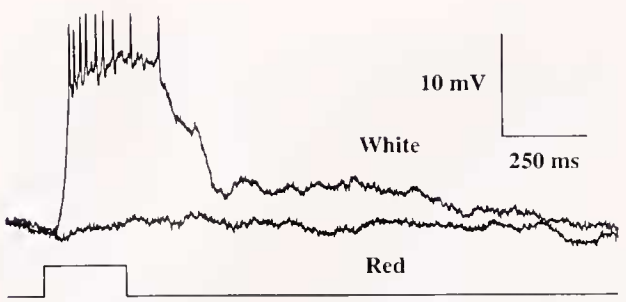

Figure 9. Intracellular receptor potential's response to white and red light (>650 nm) stimuli recorded from the same photoreceptor cell in an epistellar body. The lower trace indicates the time and duration of 250-ms light flashes. Light-flash intensity was 167 $\mu W/cm^2$ and resting potential was −53 mV in both cases.

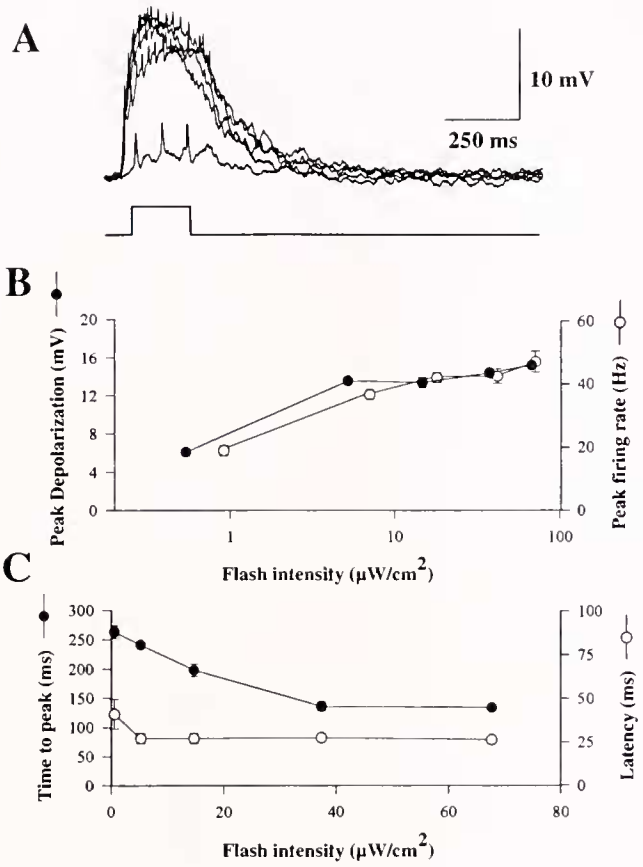

Figure 10. Typical intracellular receptor potentials recorded in a photoreceptor cell from an epistellar body, in response to change in light-flash stimulus intensity. Resting potential was −42 mV to −47 mV. (A) The upper traces show the photoreceptor potentials in response to the series of 250-ms-duration light flashes, as indicated in the lower trace in response to increased intensity. (B) Graph of light-flash intensity against response receptor potential amplitude and peak firing frequency. (C) Rise time and latency of receptor potentials against increased light stimulus intensity. In all cases each point represents the mean ± SEM, $n = 10$ separate receptor potential responses to light-flash stimuli from a single photoreceptor cell. In some cases the SEM is too small to be visible.

tric cells from *Limulus polyphemus* (Behrens and Wulff, 1965). As already seen for the extracellular compound generator potentials, the time-to-peak depolarization for individual photoreceptor cells decreased with increasing stimulus intensity (Fig. 10C). However, stimulus-to-response latency for individual photoreceptor cells was fairly constant at around 25 ms for flashes of intensity greater than 5 μW/cm^2, but longer for very weak flashes (Fig. 10C). The effect of long-duration (5 s and 10 s) but constant-intensity stimuli on the intracellular photoresponse was examined. There was an increase in the duration of the receptor potential as the light stimulus was lengthened from 5 s to 10 s, with the long-lasting plateau maintained for the duration of the stimulus and then slowly decaying back to the normal resting potential for the photoreceptor cell (Fig. 11); this probably indicates that the action potentials do not actively invade the cell soma and are thus attenuated or absent in some soma recordings.

Discussion

A number of morphological and biochemical studies have shown that octopod and decapod cephalopods have extraocular photoreceptors (reviewed in Messenger, 1991). Young (1936) first described epistellar structures in several octopod genera; from histological considerations, he hypothesized that these might have a neurosecretory function. The epistellar body of *Octopus vulgaris* was later re-investigated in detail using electron microscopy and found to contain cells that have microvilli very like those found in the rhabdomeres of the extraocular photoreceptors seen in other molluscs and arthropods (Nishioka *et al.*, 1962). This work was later extended to include the epistellar bodies of *Eledone moschata* (Nishioka *et al.*, 1966); in it, electron microscopy gave further evidence for a rhabdomeric ultrastructure, and biochemical evidence indicated the presence of the photopigment rhodopsin. These studies thus implied that the neuronlike cells of the epistellar body were not neurosecretory, but photoreceptor cells. This agreed with comparative studies showing the presence of extraocular photosensitive cells in the central nervous systems of many other invertebrates—for example, in the caudal ganglion of the crayfish (Kennedy, 1963) and the molluscs *Aplysia californica* (Arvanitaki and Chalazonitis, 1961) and *Onchidium verruculatum* (Hisano *et al.*, 1972). Preliminary electrophysiological evidence demonstrating a light-evoked response from cephalopod epistellar body photoreceptor cells was first obtained by Mauro and Baumann (1968) in an octopus, *Eledone moschata*. Decapod cephalopods such as the

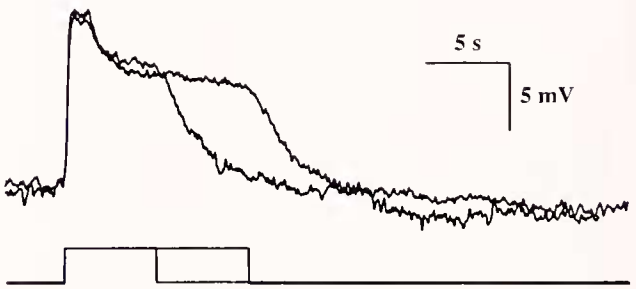

Figure 11. Intracellular receptor potentials recorded in a single photoreceptor cell from an epistellar body in response to constant intensity (77 μW/cm^2) and increased long-duration light stimuli. The receptor potentials recorded from a 5-s and 10-s light flash are shown as indicated by the stimulus marker in the bottom trace. Resting potential was −50 mV.

squid *Loligo vulgaris* and the cuttlefish *Sepia officinalis* also have PSVs, or 'parolfactory vesicles'; these are not located on the stellate ganglia, but lie underneath the cranial cartilage casing on and below the optic tract (Young, 1936; Perrelet and Mauro, 1972). These PSVs also have neuronlike cells with a rhabdomeric ultrastructure containing rhodopsin (Nishioka *et al.*, 1966; Baumann *et al.*, 1970) and their responses to light stimuli have been briefly reported for *Todarodes sagittatus* (Mauro and Sten-Knudsen, 1972; Mauro, 1977), *Loligo pealei* and *Loligo forbesi* (Sperling *et al.*, 1973; Cobb and Williamson, 1998).

Electrophysiology of extraocular photoreceptor cells

Using extracellular and intracellular recordings from extraocular photoreceptors in *Eledone cirrhosa*, the present study extends our understanding of the electrophysiology of epistellar body photoreceptors in cephalopods. The results show that the photoreceptors have cell resting potentials of about -49 ± 7 mV and respond to a flash of light with a depolarization consisting of a transient component, often accompanied by a burst of action potentials, followed by a steady-state or plateau depolarization. The amplitude of the evoked depolarization and the peak firing frequency of the cell were directly correlated with the intensity of the light flash. No 'quantum bumps' were observed in these recordings, although these have been reported in recordings from the extraocular photoreceptors of *Eledone moschata* (Mauro and Baumann, 1968), as well as from other invertebrates (Lisman and Brown, 1975).

The extracellular generator potentials recorded from the epistellar body of *Eledone cirrhosa* showed adaptational changes when the level of ambient illumination was increased in this study. This agrees well with the adaptational changes seen in retinal photoreceptors for cephalopods (Weeks and Duncan, 1974) and other invertebrates (Laughlin, 1989). It should be noted that there are no reports of screening pigments in cephalopod extraretinal photoreceptors, so the mechanism of screening pigment migration cannot be invoked here. The observed decrease in the stimulus-to-response latency of the evoked intracellular depolarization (from about 40 ms to 25 ms) with increasing intensity is similar to that seen in photoreceptor latency measurements from the epistellar bodies of both *Eledone moschata* and *Eledone cirrhosa* (Mauro, 1977; Cobb *et al.*, 1995b) and also from the retina of *Sepiola atlantica*, a sepiolid cephalopod (Duncan and Weeks, 1973). The small differences between the latency values reported in this study (40 ms) and in the others (30 and 23 ms, respectively) are probably due to the different stimulus intensities used, for as shown above, the onset delay decreases with stimulus intensity.

When two extraocular photoreceptor cells from the same cluster in a parolfactory vesicle from a squid (*Loligo pealei*) were impaled simultaneously, partial electrical coupling was recorded in 1 out of 4 pairs of cells (Sperling *et al.*, 1973). In the present study, which used extraocular photoreceptor cells from the epistellar body of an octopus (*Eledone cirrhosa*), no evidence of Lucifer yellow dye coupling was observed. The lack of dye coupling may perhaps indicate a lack of electrical coupling, for the two are often well correlated, as, for example, in the horizontal cells of the turtle retina (Stewart, 1978) and in the pineal gland photoreceptors of teleost fish, where numerous gap junctions are present (Omura, 1984), and Lucifer yellow dye coupling has been observed (Nakamura *et al.*, 1986). However, electrical coupling is not always associated with dye coupling, particularly in molluscan preparations (Williamson, 1989; Ewadinger *et al.*, 1994).

Light-flash stimuli transmitted through the red filter (Kodak 1A, wavelength >650 nm) neither stimulated the PSVs from *Eledone cirrhosa* nor evoked an afferent response in the mantle connective nerve; therefore, this wavelength of red light (>650 nm), at the intensities used in this study, did not stimulate the photoreceptors. This outcome was perhaps to be expected, for the photoreceptors in both the retinal and extra-retinal systems of *Eledone moschata* have a rhodopsin absorption maximum around 470 nm (Nishioka *et al.*, 1966; Hamdorf *et al.*, 1968), and the red light stimuli are therefore likely to be outside the detection range of the photoreceptors, at the stimulus intensities employed here.

Innervation of the epistellar body

Orthodromic fills of the photoreceptor axons with fluorescent Di-1 from the epistellar body of *Eledone cirrhosa* have shown nerve bundles passing from the epistellar body into the mantle connective (pallial) nerve. Microanatomical studies, silver-staining techniques, and electron microscopy were used in studies of the octopuses *Eledone moschata* and *Octopus vulgaris* to trace the 'epistellar nerve' from the photoreceptor cells of the epistellar body, through the stellate ganglion, to the mantle connective (pallial) nerve (Young, 1936; Cazal and Bogoraze, 1944; Nishioka *et al.*, 1966; Perrelet and Mauro, 1972). In addition, degeneration of nerve fibers running to the epistellar body was shown in *E. moschata* after the mantle connective nerve was cut (Young, 1936). The mantle connective (pallial) nerve is known to contain about 16,000 nerve fibers (Young, 1965), with two groups of efferent, and possibly two groups of afferent fibers (Young, 1971). In *E. moschata*, the epistellar nerve contains about 1500 afferent nerves (Perrelet and Mauro, 1972). Therefore, the selective nerve tracing in this study (using Di-1 and electrophysiological recordings from the mantle connec-

tive (pallial) nerve) presents new evidence supporting the view that the epistellar nerve contains the afferent axons of extraocular photoreceptor cells, and that these axons conduct photoreceptive information to the central nervous system in the octopus. Whether such sensory information passes to the palliovisceral lobe of the octopus brain from nerves carried in the mantle connective (pallial) nerve remains to be determined.

In summary, this study provides further evidence for the presence of an active extraocular photoreceptor system in adult cephalopod molluscs. However, the functional role of the epistellar body and parolfactory vesicles in octopus and squid still remains enigmatic. The lack of a structurally organized retina or optical apparatus such as a lens, and the relatively deep location of all these PSVs argues against any function in visual image formation. Rather, it is generally assumed that in both octopods and decapods the extraocular photoreceptors play a role in monitoring ambient light levels over lengthy periods; that is, they function as a photometer (Mauro, 1977; Houck, 1977a,b), perhaps connected with seasonal reproductive activity (Baumann *et al.*, 1970), control of circadian activity rhythms in octopods (Houck, 1977ab, 1981, 1982), diel vertical migration in squid (Palmer and O'Dor, 1978), or even the feedback control of ventral photophores in midwater squid such as *Abralia trigonura* (Young, 1972, 1973; Young *et al.*, 1979).

Observations increasingly suggest that the influences of environmental light on the physiology and behavior of cephalopods may be detected not only by the eye, but also by an array of other photoreceptor organs, including the PSVs. Some of these photoreceptors still remain to be studied in detail—those located on the head of hatchling cuttlefish and squid (Sundermann, 1990), for example. Whether it is such extraocular receptors or the ocular sensory pathways that control such behavior as 'search' or 'avoidance' reactions to light (*e.g.*, in juvenile cephalopods) has not yet been examined.

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